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Request for grant of a patent

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1. Your reference — SMC 60565/GB/P1

2. Patent application number *(The Patent Office will fill in this part)* — 0229443.7 — 18 DEC 2002

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Avecia Limited
Hexagon House
Blackley
Manchester, M9 8ZS

Patents ADP number *(if you know it)* 07764137001

If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom

4. Title of the invention — Process

5. Name of your agent *(if you have one)* — REVELL, Christopher

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Avecia Limited
Hexagon House
PO Box 42
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Patents ADP number *(if you know it)* [redacted] 07764137002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application.

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

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b) there is an inventor who is not named as an applicant, or

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Pate Form 1/77

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Continuation sheets of this form	
Description	11
Claim(s)	1
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1. I/We request the grant of a patent on the basis of this application.

Signature [signature] Date 18/12/98

Avecia Limited Authorised Signatory

2. Name and daytime telephone number of person to contact in the United Kingdom: K.M.Pinder/G.Terry 0161 721 1361/2

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PROCESS

The present invention concerns a process for the preparation of oligonucleotides.

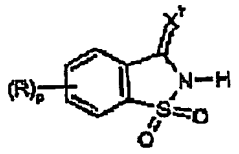
Oligonucleotides are conventionally prepared by solid phase synthesis wherein the nascent oligonucleotide is coupled to a solid support. Conventionally, phosphoramidite chemistry in the presence of a tetrazole or substituted tetrazole activator is employed to effect the sequential coupling of nucleosides. The solid supports employed in the majority of synthetic applications are rigid, non-swellable supports, particularly controlled pore glass and rigid polystyrene. A number of attempts have been made to employ swellable supports, which offer advantages in terms of much higher potential loadings. These too have employed conventional tetrazole-based activators. Generally, the results have been disappointing, giving either slow coupling reactions or requiring very high amounts of activator.

Surprisingly, it has now been found that the selection of a particular class of activators enables significantly improved synthesis of oligonucleotides using phosphoramidite chemistry and swellable supports.

According to the present invention, there is provided a process for the synthesis of an oligonucleotide in which an oligonucleotide is assembled on a swellable solid support using the phosphoramidite approach in the presence of an activator, wherein the activator is not tetrazole or a substituted tetrazole.

Activators which can be employed in the process of the present invention include salts of heteroaromatic compounds comprising fewer than four nitrogen atoms in the heteroaromatic ring, especially heteroaromatic compounds comprising a 5 or 6 membered ring which comprises one or two nitrogen atoms. Examples include pyridinium, imidazolinium and benzimidazolinium salts, particularly the hexafluorophosphate, tetrafluoroborate, triflate, hydrochloride, trifluoroacetate, dichloroacetate, O-mesyl, O-tosyl, bromide or trifluorosulphonyl salts as disclosed in PCT application WO 99/62922 (incorporated herein by reference); benzotriazole and derivatives thereof, especially hydroxybenzotriazole; and saccharin or a saccharin derivative, preferably employed as a salt-complex formed with an organic base, especially the N-methylimidazole, pyridine or 3-methylpyridine salts of saccharin.

The saccharin or saccharin derivative which can be employed preferably has the general formula:



I.

In Formula I, p is 0 or an integer from 1 to 4. R for each occurrence is a substituent, preferably each independently, a halo, a substituted or unsubstituted aliphatic group, $-NR^1R^2$, $-OR^3$, $-OC(O)R^3$, $-C(O)OR^3$, cyano, a substituted or unsubstituted aryl, a substituted or unsubstituted heterocyclyl, -CHO, $-COR^3$, $-NHCOR^3$, a substituted or unsubstituted aralkyl, halogenated alkyl (e.g., trifluoromethyl and trichloromethyl), or $-SR^3$. Preferably, R is halo, a substituted or unsubstituted aliphatic group, $-NR^1R^2$, $-OR^3$, $-OC(O)R^3$, $-C(O)OR^3$, or cyano. Alternatively, two adjacent R groups taken together with the carbon atoms to which they are attached form a six membered saturated or unsaturated ring. Preferably, the six membered ring formed is an aromatic ring. R^1 and R^2 are each, independently, -H, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl group; or together with the nitrogen to which they are attached form a heterocyclyl group. R^3 is a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, or a substituted or unsubstituted aralkyl group. X is O or S. Preferably, X is O. It is particularly preferred that X is O and p is 0.

Suitable substituents which may be present include aryl groups, halogenated aryl groups, alkyl groups, halogenated alkyl (e.g. trifluoromethyl and trichloromethyl), aliphatic ethers, aromatic ethers, benzyl, substituted benzyl, halogens, particularly chloro and fluoro groups, cyano, nitro, -S-(aliphatic or substituted aliphatic group), and -S-(aromatic or substituted aromatic) groups.

Preferably the saccharin or saccharin derivative is employed as a salt complex with an organic base.

Organic bases which can form salt-complexes with saccharin or saccharin derivatives are organic compounds that have a tendency to accept protons at pH 7. Preferred organic bases are secondary amines, tertiary amines or azaheterocyclyl bases, each of which may be substituted or unsubstituted by one or more substituents. An aprotic organic base is an organic base that has no hydrogen bonding protons in its chemical structure before accepting a proton. Aprotic organic bases such as tertiary amines and aprotic azaheterocyclyl compounds are preferably used in conjunction with compounds of formula 1, as described herein.

Azaheterocyclyl bases, as used herein, include heteroaryl groups which have one or more nitrogen atom in the aromatic ring and heteroalicyclyl groups that have at least one nitrogen atom in the non-aromatic ring system. Preferably, azaheteroaryl bases have five- or six-membered aromatic rings with from one to three nitrogens in the aromatic ring. Preferably, azaheteroalicyclyl compounds are five- or six-membered rings, commonly comprising one or two nitrogens in the ring. Examples of azaheterocyclyl bases include pyrimidines, 1-alkylpyrazoles, especially 1-(C_{1-4} alkyl)pyrazoles, 1-arylpyrazoles, 1-benzylpyrazoles, pyrazines, N-alkylpurines, especially N-(C_{1-4} alkyl)purines, N-arylpurines, N-benzylpurines, N-alkylpyrroles, especially N-(C_{1-4} alkyl)pyrroles, N-arylpyrroles, N-

benzylpyrroles, pyridines, N-alkylimidazoles, especially N-(C_{1-4} alkyl)imidazoles, N-arylimidazoles, especially N-phenylimidazole, N-benzylimidazoles, quinolines, isoquinolines, quinoxalines, quinazolines, N-alkylindoles, especially N-(C_{1-4} alkyl)indoles, N-arylindoles, N-benzylindoles, N-alkylbenzimidazoles especially N-(C_{1-4} alkyl)benzimidazoles, N-arylbenzimidazoles, N-benzylbenzimidazoles, triazine, thiazole, 1-alkyl-7-azaindoles, especially 1-(C_{1-4} alkyl)-7-azaindoles, 1-aryl-7-azaindoles, 1-benzyl-7-azaindoles, pyrrolidines, morpholines, piperidines, and piperazines. Especially preferred azaheterocyclyl bases are pyridines, such as pyridine and 3-methylpyridine, and N-(C_{1-4} alkyl) imidazoles, such as N-methylimidazole.

Tertiary amines are organic bases that have a nitrogen atom which is bonded to three carbon atoms, often to three aryl, commonly phenyl, and/or alkyl groups, commonly to three alkyl groups, including for example trialkylamines such as trimethylamine, triethylamine, and diisopropylethylamine. In addition, tertiary amines can be azaheterocyclyl groups wherein the nitrogen atom is aprotic. Tertiary amines that are azaheterocyclyl groups are preferred. Examples of azaheterocyclyl tertiary amines are N-alkylpyrrolidines, N-arylpyrrolidines, N-alkylpyrroles, N-arylpyrroles, N-alkylmorpholines, N-arylmorpholines, N-alkylpiperidines, N-arylpiperidines, N,N-dialkylpiperazines, N,N-diarylpiperazines, N-alkyl-N-aryl-piperazines, quinuclidines, 1,5-diazabicyclo[4.3.0]non-5-enes and 1,8-diazabicyclo[5.4.0]undec-7-enes. Tertiary amines can also be azaheteroaryl or azaheteroalicyclyl compounds.

Secondary amines are organic bases comprising a nitrogen bonded to a single hydrogen and to two carbon atoms. Commonly the nitrogen atom is bonded to two alkyl or aryl groups or forms part of an azaheterocyclic group. Examples of secondary amine compounds include diethylamine and diisopropylamine.

Particularly preferred organic bases include pyridine, 3-methylpyridine, and N-methylimidazole.

Swellable solid supports which can be employed in the process according to the present invention are those which increase in volume when contacted with an appropriate solvent. It will be recognised that the extent of the swelling will vary from support to support, and depending on the nature of the solvent. Preferred swell ratios for a swellable solid support falls in the range of from 5 to 20. The swell ratio is defined as :-

$$\text{Swell Ratio} = \frac{Vol_{final} - Vol_{initial}}{Vol_{initial}}$$

Vol_{final} = Final volume occupied by support after allowing the support to fully swell in a given solvent.

$Vol_{initial}$ = Initial dry bed volume of support.

Swellable solid supports are typically cross linked polymers wherein the amount of cross-linking is low enough to permit swelling. The extent of crosslinking in these polymers can be expressed in percentage terms and corresponds to the number of moles of polymerisable double bonds derived from monomers containing two or more polymerisable double bonds as a percentage of the total number of moles of polymerisable double bonds. The percentage of cross linking is often in the range of from 0.1, commonly from 0.5, to 20%, such as from 1 to 10%, and preferably no more than 5%. Polymers comprising no more than 20% of cross-linking are generally swellable, whilst polymers comprising greater than 20% of crosslinking are generally not swellable. Most preferably a level of crosslinking from 1% to 5%, especially from 1% to 3% is employed.

The polymer support may be derived from the polymerisation of a composition comprising one or more monomers, and is preferably derived from the polymerisation a composition comprising of two or more monomers. The monomers may contain one or more polymerisable double bonds. Preferably the polymer support is derived from the polymerisation of a composition comprising one or more monomers containing only one polymerisable double bond, and one or more monomers containing two or more polymerisable double bonds. Most preferably the polymer support is derived from the polymerisation of a composition comprising one or two monomers containing only one polymerisable double bond, and one monomer containing two or three polymerisable double bonds.

Examples of monomers containing only one polymerisable double bond include styrene and substituted styrenes such as α-methyl styrene, methyl styrene, t-butyl styrene, bromo styrene and acetoxy styrene; alkyl esters of mono-olefinically unsaturated dicarboxylic acids such as di-n-butyl maleate and di-n-butyl fumarate; vinyl esters of carboxylic acids such as vinyl acetate, vinyl propionate, vinyl laurate and vinyl esters of versatic acid such as VeoVa 9 and VeoVa 10 (VeoVa is a trademark of Shell); acrylamides such as methyl acrylamide and ethyl acrylamide; methacrylamides such as methyl methacrylamide and ethyl methacrylamide; nitrile monomers such as acrylonitrile and methacrylonitrile; and esters of acrylic and methacrylic acid, preferably optionally substituted C_{1-20}alkyl and C_{1-20}cycloalky esters of acrylic and methacrylic acid, such as methyl acrylate, ethyl acrylate, n-butyl acrylate, 2-ethylhexyl acrylate, i-propyl acrylate, and n-propyl acrylate, methyl methacrylate, ethyl methacrylate, n-butyl methacrylate, 2-ethylhexyl methacrylate, i-propyl methacrylate, n-propyl acrylate, hydroxyethyl acrylate, hydroxyethyl methacrylate, N,N-dimethylaminoethyl acrylate and N,N-dimethylaminoethyl methacrylate. Functional derivatives of the foregoing monomers containing only one polymerisable double bond can also be employed.

Examples of monomers containing two or more polymerisable double bonds include divinylbenzene (DVB), trivinylbenzene, and multifunctional acrylates and methacrylates such as ethylene glycol diacrylate, ethylene glycol dimethacrylate,

trimethylolpropane triacrylate, trimethylolpropane trimethacrylate, ethylene bisacrylamide, pentaerythritol triacrylate, pentaerythritol tetraacrylate, pentaerythritol trimethacrylate, pentaerythritol tetramethacrylate and N,N-bis-acryloyl ethylene diamine. Preferably the cross-linking monomer, particularly for the preparation of cross-linked polystyrene, is DVB.

Preferred examples of swellable supports include copolymers comprising polystyrene such as polystyrene-poly(ethylene glycol) copolymers, functionalised polystyrenes, especially polystyrenes funtionalised with polyethylene glycols, including those polymers disclosed in WO00/02953, incorporated herein by reference, polystyrene which is graft-copolymerised with polyethyleneglycol, such as those polymers available under the trade name "Tentagel" which comprise a polystyrene core with polyethylene glycol (MWt ca 4000) chains grafted onto this core, and polymers such as polyvinylacetate. Further preferred supports include partially-hydrolysed poly(vinylacetate). Additionally, poly(acrylamide) supports, especially microporous or soft gel supports, such as those more commonly employed for the solid phase synthesis of peptides may be employed if desired. Preferred poly(acrylamide) supports are amine-functionalised supports, especially those derived from supports prepared by copolymerisation of acryloyl-sarcosine methyl ester, N,N-dimethylacrylamide and bis-acryloylethylenediamine, such as the commercially available (Polymer Laboratories) support sold under the catalogue name PL-DMA. The procedure for preparation of the supports has been described by Atherton, E.; Sheppard, R. C.; in *Solid Phase Synthesis: A Practical Approach*, Publ., IRL Press at Oxford University Press (1984) which is incorporated herein by reference. The functional group on such supports is a methyl ester and this is initially converted to a primary amine functionality by reaction with an alkyl diamine, such as ethylene diamine.

The swellable solid supports comprise a functional group on which oligonucleotide synthesis can be effected. Examples of functional group are amino and hydroxy groups.

The oligonucleotide synthesis can take place by direct attachment to the functional group of the solid support. However, in many embodiments, it is preferred to employ a cleavable linker to attach the oligonucleotide to the solid support via the functional group. Examples of such linker are well known in the art and include particularly succinyl, oxaloyl and trityl linkers.

In many embodiments, the support is swelled in the solvent of choice to allow ready access to the functional groups on the support. Solvents of choice can be predicted by considering the polymer composition and are often those solvents which would be "good solvents" for a theoretical linear polymer which may be made from a similar composition but with no crosslinking agent present.

The process of the present invention preferably employs a solvent which is selected to swell the solid support. It will be recognised that the nature of the solvent will be selected based upon the nature of the solid support employed. Examples of suitable

solvents suitable for use in phosphoramidite chemistry are well known in the art, and include in particular acetonitrile, dimethylformamide, N-methylpyrrolidinone, dichloromethane, THF and pyridine.

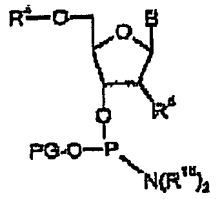
Oligonucleotides that can be prepared by the process of the present invention include oligodeoxyribonucleotides, oligoribonucleoside, and oligonucleotides comprising mixtures of deoxyribo- and ribonucleosides. The oligonucleotides may be modified by one or more modifications known in the field of oligonucleotide chemistry, for example ribonucleoside moieties may be modified at one or more of the 2'-positions by the presence of 2'-alkoxy group, such as a methoxy or methoxyethoxy group. Deoxyribonucleosides moieties may be modified at the 2'-position by the presence of a substituent, such as a halo group, especially a fluoro group, or by an alkenyl group such as an allyl group. Abasic nucleoside moieties may also be present. One or more locked nucleoside may be present. In many embodiments, the oligonucleotides will be in the form of the natural D-isomer. However, some or all of the oligonucleotide may represent an unnatural isomer, for example an L-isomer or a B-anomer, either in whole or in part. The internucleoside linkages may be natural phosphate, or one or more modified linkages, for example phosphorothioate or phosphoramidate linkages may be present.

The oligonucleotide may comprise one or more protecting groups. Examples of such protecting groups, and the positions which they can be employed to protect, are well known to those skilled in the art, and include trityl, monomethoxytrityl and dimethoxytrityl groups, levulinoyl groups, isobutyryl groups, benzoyl groups, acetyl groups and carbonate groups, such as BOC and especially FMOC.

The oligonucleotides may comprise natural and/or unnatural nucleobases including adenine, guanine, cytosine, thymine, uracil, 7-deazaguanine, 7-deaza-8-azaguanine, 5-propynylcytosine, 5-propynyluracil, 7-deazaadenine, 7-deaza-8-azaadenine, 7-deaza-6-oxopurine, 6-oxopurine, 3-deazaadenosine, 2-oxo-5-methylpyrimidine, 2-oxo-4-methylthio-5-methylpyrimidine, 2-thiocarbonyl-4-oxo-5-methylpyrimidine, 4-oxo-5-methylpyrimidine, 2-amino-purine, 5-fluorouracil, 2,6-diaminopurine, 8-aminopurine, 4-triazolo-5-methylthymine, 4-triazolo-5-methyluracil and hypoxanthine.

The oligonucleotide is preferably prepared by coupling a deoxyribonucleside-3'-phosphoramidite or ribonucleside-3'-phosphoramidite with a nascent oligonucleotide comprising a free 5'-hydroxy group. However, it will be recognised that the process according to the present invention is equally applicable to the coupling of a 5'-phosphoramidite to 3 free 3'-hydroxy group.

Preferred phosphoramidites are compounds of formula:



wherein R^4 is a protecting group, preferably a trityl, monomethoxytrityl or dimethoxytrityl group, B is a nucleoside base, R^5 represents -H, -F -OR^6, -NR^7R^8, -SR^9, or a substituted or unsubstituted aliphatic group, such as methyl or allyl. PG is a phosphorus protecting group, commonly a cleavable phosphorus protecting group employed in oligonucleotide synthesis, and preferably a substituted or unsubstituted aliphatic group or a group of formula -OCH_2CH_2CN, -SCH_2CH_2CN, -OR^{11}, -SR^{11}, -O-CH_2CH_2-$Si(CH_3)_2C_6H_5$, -O-CH_2CH_2-$S(O)_2$-CH_2CH_3, -O-CH_2CH_2-C_6H_4-NO_2, -S-CH_2CH_2-$Si(CH_3)_2C_6H_5$, -S-CH_2CH_2-$S(O)_2$-CH_2CH_3, or -S-CH_2CH_2-C_6H_4-NO_2. R^6 represents -H, a substituted or unsubstituted aliphatic group (e.g., methyl, ethyl, methoxyethyl or allyl), a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl, an alcohol protecting group, especially a base-labile or a silyl protecting group, or -$(CH_2)_q$-$NR^{12}R^{13}$. R^7 and R^8 are each, independently, -H, a substituted or unsubstituted aliphatic group, or an amine protecting group. Alternatively, R^7 and R^8 taken together with the nitrogen to which they are attached are a heterocyclyl group. R^9 represents -H, a substituted or unsubstituted aliphatic group, or a thiol protecting group. R^{11} represents a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group or a substituted or unsubstituted aralkyl group. R^{12} and R^{13} are each, independently, -H, a substituted or unsubstituted aryl group, a substituted or unsubstituted heteroaryl group, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aralkyl group, a substituted or unsubstituted heteroaralkyl group or an amine protecting group. Alternatively, R^{12} and R^{13} taken together with the nitrogen to which they are attached form a heterocyclyl group. q is an integer from 1 to about 6. Each R^{16} independently is a C_{1-6} alkyl group, preferably an isopropyl group. The phosphoramidite employed is commonly a betacyanoethyloxy-N,N-diisopropyl phosphoramidite.

The process according to the present invention may employ such process steps as are conventionally carried out for the solid-phase synthesis of oligonucleotides using phosphoramidite chemistry, including sulfurisation, oxidation and capping stages.

When a sulphurization agent is employed, preferably the sulfurization agent is an organic sulfurization agent.

Examples of organic sulfurization agents include 3*H*-benzodithiol-3-one 1,1-dioxide (also called "Beaucage reagent"), dibenzoyl tetrasulfide, phenylacetyl disulfide, N,N,N',N'-tetraethylthiuram disulfide, elemental sulfur, and 3-amino-[1,2,4]-dithiazole-5-thione (see U.S. Patent No. 6,096,881, the entire teachings of which are incorporated

herein by reference).

Typical reaction conditions for sulfurization of an oligonucleotide using the above agents can be found in Beaucage, *et al.*, *Tetrahedron* (1993), *49*:6123, which is incorporated herein by reference.

Preferred sulfurization reagents are 3-amino-[1,2,4]-dithiazole-5-thione and phenylacetyl disulfide.

Sulfurization of an oligonucleotide may be carried out by, for example use of a solution of 3-amino-[1,2,4]-dithiazole-5-thione in an organic solvent, such pyridine/acetonitrile (1:9) mixture or pyridine, having a concentration of about 0.05 M to about 0.2 M.

Examples of oxidising agents which may be employed include iodine and peroxides, such as t-butylhydroperoxide.

A desired oligonucleotide can be prepared for example by a sequence of steps which comprise coupling a protected, commonly a 5'-protected, nucleoside phosphoramidite with a free hydroxy group, oxidising or sulfurising the protected phosphite triester formed in the coupling step to form a phosphate or phosphorothioate oligonucleotide, removing the protecting group from the oligonucleotide, and repeating the cycle until the desired sequence has been assembled. The oligonucleotide can be cleaved from the solid support, and any remaining protecting groups, such as nucleobase and phosphorus protecting groups can be removed using conditions known in the art.

The process according to the present invention can be carried out in a wide range of appropriate reaction vessels, including, for example, columns, stirred vessels and fixed bed reactors.

The present invention is illustrated without limitation by the following example.

Example

Synthesis of DMTrOABz-3'-Succinate

DMTrOABzOH (75.0 g, 117 mmol) was charged to an oven-dried 500ml florentine, followed by succinic anhydride (15.6 g, 160 mmol) and *N,N*-dimethylaminopyridine. The flask was flushed with nitrogen gas and the neck was fitted with a rubber septum. Anhydrous pyridine (250 ml) was charged to the flask *via* syringe. The resulting solution was stirred at room temperature for 82 hours after which the bulk of the pyridine was removed *in vacuo*. The resulting crude oil was stored in a stoppered flask for 60 hours.

The oil was dissolved in DCM (250 ml) and this solution was washed with water (2 x 250 ml) and triethylammonium phosphate solution (3 M, pH 7.5, 250 ml). The organic layer was separated, dried with $MgSO_4$ and the solvent was removed *in vacuo*. The residue was dissolved in toluene (200 ml) and the solvent was removed *in vacuo*. This was repeated once more with toluene (200 ml) and once with DCM (200 ml). This yielded an

off-white foam which, after 3 hours drying under vacuum, could be crushed to a free-flowing powder (94.4 g).

The strength of this product was measured (HPLC, area %) as 97 %, corresponding to a yield of 91 %.

Preparation of HOABz-3'-succinate-polystyrene resin

Resin: ex Novabiochem, 1.13 mmolg^{-1} aminomethylated polystyrene resin (1 % crosslinked DVB), cat. no. 01-64-0010.

Aminomethylated polystyrene resin (1% crosslinked with divinylbenzene) obtained from Novabiochem (cat. no. 01-64-0010) (5 g, "5.65 mmol", 1 eq) was placed in a large straight-edged sinter funnel and pre-swollen with N-methylpyrrolidinone (NMP, ca. 30 ml), applying a positive pressure of nitrogen *via* a side-arm to bubble through the thick paste. The NMP was discharged after ca. 10 minutes.

DMTrOABz succinate (19.4 g, 3.9 eq) and hydroxybenzotriazole (HOBt) (4.6 g, 6 eq) were dissolved in NMP (ca. 35 ml) and diisopropylcarbodiimide (DIC, 2.1 g, 2.9 eq) was added to this solution. Diisopropylethylamine (1.7 g, 3 eq) was added to this solution ca. two minutes after the DIC had been added. This whole solution was swirled then quickly added to the swollen resin in the sinter, again with a positive pressure of nitrogen from below the sinter providing agitation for the thick yellow gel. Glassware and equipment contaminated with DIC was detoxified in a caustic bath.

The sinter funnel was covered over to prevent contamination of the reaction mixture (non-gastight seal) and the reaction was left bubbling gently for 65 hours.

Checking the progress of the reaction by standard Kaiser tests gave a negative result (yellow colour) after this period and the reaction was deemed complete.

The reagent solution was discharged by swapping the N_2 flow for a vacuum and sucking the solution into the flask. The resin was washed with NMP (3 x ~ 40 ml). Any remaining free amino groups were capped by adding a solution of acetic anhydride (4.2 g, 7.2 eq) and N,N-dimethylaminopyridine (0.07 g, 0.1 eq) in NMP (~ 35 ml) and holding for 1 hour. The reagents were discharged and the resin was washed with dimethylformamide (DMF, 5 x 40 ml), dichloromethane (DCM, 5 x 40 ml) and was finally collapsed with diethyl ether (3 x 40 ml). The resin was blown dry with a stream of nitrogen and dried in a vacuum oven at room temperature overnight. The spent reagent solution was treated with NaOH solution for detoxification.

The resin (1.028 g) was charged to a jacketed phase reactor and washed with DCM (~ 10 ml). The reactor jacket was cooled to 0°C with a fluid circulator. While suction was applied, 3 % v/v dichloroacetic acid solution in DCM (84 ml) was charged to the reactor. A deep red colour developed immediately. Once all of the acid solution had passed through the resin bed (110 s) the resin was washed with DMF (5 x ~ 10 ml) and DCM (5 x ~ 10 ml) and finally collapsed with diethyl ether (3 x ~ 20 ml).

Preparation of AcOG(Isobu)TABzCBzABzPS

For all "dry" steps in coupling reactions (i.e. pre-coupling washes to sulfurization) the DMF used was commercial anhydrous DMF (ex Aldrich), which was dried overnight over molecular sieves in ca. 100 ml batches as required. This gave DMF with a moisture content of 10-50 ppm (Karl-Fischer), c.f. ~150 ppm as initially supplied. Discharging solutions was achieved in these steps by applying a positive pressure of N_2 gas to the top of the reactor *via* the Rotaflo tap. The resin used initially in this series of reactions was 0.5 $mmol g^{-1}$ of the HOABz-polystyrene resin prepared above. All phosphoramidites were protected deoxyribo-3'-betacyanoethyloxy-N,N-diisopropylphosphoramidites.

The HOABz-polystyrene resin (1.356 g, 0.69 mmol) was charged to the jacketed solid-phase reactor, which was fitted with a septum inlet and a Rotaflo tap. Both the nitrogen inlet and outlet were fitted with in-line drying tubes filled with self-indicating P_2O_5. The resin was washed with dry DMF (3 x ~ 5 ml) and dry DCM (2 x ~5 ml). The amidite (1.41 g, 2.5 eq) was dried azeotropically with MeCN (2 x 10 ml) and dissolved in dry DCM (~ 3 ml). N-methylimidazole salt of saccharin (0.45 g, 2.5 eq) was charged to an oven-dried vial fitted with a septum and dissolved in dry DMF (~0.75 ml) and dry DCM (~1.5 ml). The amidite and saccharin salt solutions were then charged to the pre-swollen resin, in that order.

After a two hour hold with gentle bubbling of the mixture with N_2 gas, dry methanol (~2 ml) was added. After ca. 5 minutes the solution was discharged from the reactor and the resin was washed with dry DMF (3 x ~ 5 ml) and dry pyridine (2 x ~ 5 ml). The spent reagent solution was analysed by HPLC to estimate the amount of active amidite remaining at the end of reaction.

A solution of 3-amino-[1,2,4]-dithiazole-5-thione (0.25 g, 2.5 eq) in dry pyridine (~ 4 ml) was charged to the resin and this was held, with gentle N_2 bubbling, for one hour after which the solution was discharged. The top was removed from the reactor and the resin was washed with bench DMF (5 x ~ 5ml) and DCM (5 x ~ 5 ml) and then with Cap A solution (5:3:2 MeCN:Py:NMI, 2 x ~ 5ml). Cap A solution (2.5 ml) and Cap B solution (4:1

MeCN:Ac_2O, 2.5 ml) were then charged to the reactor and the mixture was held for one hour.

The spent capping solution was discharged and the resin was then washed with DMF (5 x ~ 5 ml) and DCM (5 x ~ 5 ml) and finally with diethyl ether (3 x ~ 5 ml). The resin was then left overnight before detritylation.

Prior to detritylation the reactor jacket was cooled to 0 °C and the resin was washed/pre-swollen with DCM. The resin was held under suction while a 3 % dichloroacetic acid solution (in DCM) was passed through the bed, causing a deep red colouration to appear. The volume used was based on 100 ml of 3 % acid solution per 1 mmol DMT expected to be attached to the resin. Once all the acid solution had passed through the resin bed the resin was washed with DMF (5 x ~ 5 ml) and DCM (5 x ~ 5 ml). A further 1 volume of the acid solution was passed through the resin, taking on a pale orange colour as it did so. The resin was again washed with DMF (5 x ~ 5 ml) and DCM (5 x ~ 5 ml) and finally with diethyl ether (3 x ~ 5 ml) in readiness for the next coupling reaction.

After coupling the HOABzPS resin sample with dC, dA, T and dG amidites, using the same procedure each time and capping after the final detritylation step, the AcOG(isobu)TABzCBzABZPS resin was found to weigh 2.653 g.

CLAIMS

1. A process for the synthesis of an oligonucleotide in which an oligonucleotide is assembled on a swellable solid support using the phosphoramidite approach in the presence of an activator, wherein the activator is not tetrazole or a substituted tetrazole.

2. A process according to claim 1, wherein the activator is selected from the group consisting of pyridinium, imidazolinium and benzimidazolinium salts, particularly hexafluorophosphate, tetrafluoroborate, triflate, hydrochloride, trifluoroacetate, dichloroacetate, O-mesyl, O-tosyl, bromide or trifluorosulphonyl salts; benzotriazole and derivatives thereof, especially hydroxybenzotriazole; and saccharin or a saccharin derivative, preferably employed as a salt-complex formed with an organic base, especially the N-methylimidazole, pyridine or 3-methylpyridine salts of saccharin.

3. A process according to either preceding claim, wherein the swellable support comprises polystyrene, partially hydrolysed polyvinylacetate and poly(acrylamide).